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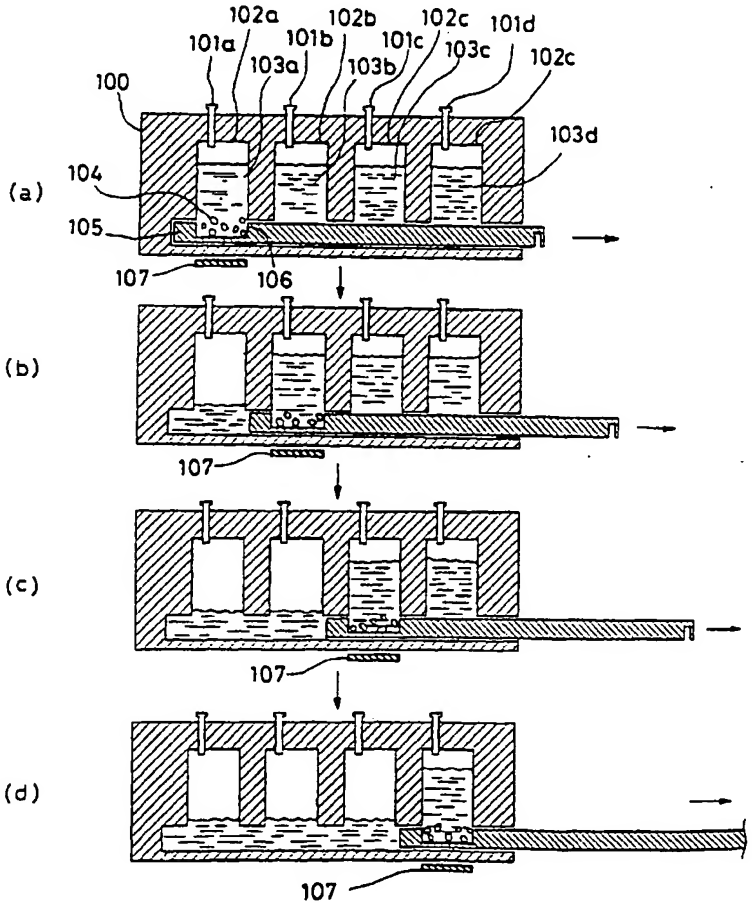
(54) Title: APPARATUS FOR ISOLATING NUCLEIC ACID OR BIOLOGICAL MATERIAL



(57) Abstract: The present invention provides an apparatus for isolating nucleic acid or biological material from biological sample using a solid material that carries or absorbs nucleic acid or biological material, the apparatus comprising: a cassette in which plural chambers and input holes into the respective chambers are formed and the plural chambers are connected through a slot which is formed in the bottom of the chambers; a slider which is fitted through the slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the slider can be pulled back and forth according to the length of the slot; a cavity which is formed on the chamber side of the slider to settle or collect a solid material in the chamber; a means for pulling the slider to transfer the solid material from one chamber to the next chamber; and a means for agitating the liquid reactants and the solid material in the plural chambers.

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APPARATUS FOR ISOLATING NUCLEIC ACID OR BIOLOGICAL MATERIAL

FIELD OF THE INVENTION

The present invention relates to an apparatus for isolating and purifying nucleic acid, cell and various biological materials.

BACKGROUND OF THE INVENTION

Isolation of nucleic acid or biological materials from various biological samples is important starting step in the fields of many areas such as biology, biochemistry, molecular biology, forensic medicine, medical diagnostic and etc. Traditional method for isolating nucleic acid involves harmful organic solvents such as phenol and chloroform. (Sambrook, J., E. F. Fritsoh and T. Maniatis 1989. Molecular cloning: A laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor. N. Y.)

Recently, several methods were provided using materials that have the proclivity of binding nucieic acid. Concrete examples of these materials are silica (Boom, R., Sol, C. J. A., Salimans, M. M. M., Jansen, C. L., Wertheim-van Dillen, P. M. E., and van der Noordaa, J. (1990) J. Clin, Microbiol. 28, 495-503), glass fibers (Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615-619, Marko, M. A., Chlpperfield, R., and Birnbolm, H. C. (1982) Anal. Biochem. 121, 382-

387), anion exchange resins (Wang, K., Gan, L., Boysen, C., and Hood, L. (1995) Anal. Biochem. 226, 85-90) and modified magnetic beads (Rudi, K., Kroken, M., Dahlherg, O. J., Deggerdal, A., Jakobsen, K. S., and Larsen, F. (1997) BioTechniques 22, 506-511, and Deggerdal, A., and Larsen, F. (1997) BioTechniques 22, 554-557).

The advantages of the method using these materials is that no harmful organic solvent is involved, and physical and biochemical degrading of nucleic acid during the isolation process is minimized. In addition, immobilized nucleic acid is less susceptible to digestion by nucleic acid degrading enzymes.

The above mentioned methods, however, still need intensive manual pipetting steps to transfer the solid material to other vessel and thus the performer is vulnerable to potential viral and bacterial infection from infectious virus and bacteria if infected blood or bacteria is starting material of nucleic acid isolation.

In order to avoid the intensive and tedious manual steps and to eliminate performer's potential error, several automatic machines such as "MagNa Pure LC" (Roche, Mannheim, Germany AG), "GENESIS" (Techan, Hombrechtikon, Switzerland) and others were developed for high number of sample manipulation. Most of them apply magnetic bead to collect nucleic acid or biological materials from various biological samples to eliminate the use of harmful chemical solvents and centrifugation step. Although these machines are adequate to continuous isolation of nucleic acid or biological material and secure high

throughput, they are expensive, rather complicate and inefficient for low and medium number of sample manipulation so that they are not practical for most research and clinical laboratories.

In light of these drawbacks of the traditional apparatus for isolating nucleic acid or biological material, there is a need for an apparatus that is efficient, inexpensive and reproducible as well as applicable to various biological samples.

SUMMARY OF THE INVENTION

A feature of the present invention is to provide an apparatus for isolating a nucleic or biological material from low and medium number of biological samples in which intensive manual pipetting step is eliminated and the risk of viral and bacterial infections from the biological samples is reduced.

Another feature of the present invention is to provide an apparatus for isolating nucleic acid or biological material from the biological samples less expensive and more readily available to researchers, medical personnel and other potential users of the technology.

According to the one aspect of the present invention, there is provided an apparatus for isolating nucleic acid or biological material from biological sample using a solid material which carries or absorbs nucleic acid or biological material, the apparatus comprising:

(a) a cassette in which plural chambers and input holes into the respective chambers are formed and the plural chambers are

connected through a slot which is formed in the bottom of the chambers;

(b) a slider which is fitted through the slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the slider can be pulled back and forth according to the length of the slot,;

(c) a cavity which is formed on the chamber side of the slider to settle or collect a solid material in the chamber;

(d) a means for pulling the slider to transfer the solid material from one chamber to the next chamber; and

(e) a means for agitating the liquid reactants and the solid material in the plural chambers.

According to the another aspect of the present invention, there is provided an apparatus for isolating nucleic acid or biological material from biological sample using a solid material which carries or absorbs nucleic acid or biological material, the apparatus comprising:

(f) a cassette in which plural chambers and input holes into the respective chambers are formed, the bottoms of the plural chambers are connected through a first slot and the tops of the plural chambers are connected through a second slot;

(g) a first slider which is fitted through the first slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the first slider can be pulled back and forth according to the length of the first slot,;

(h) a cavity which is formed on the chamber side of the first slider to settle or collect a solid material in the chamber;

(i) a second slider having plural intruders toward the plural chamber to agitate the liquid reactants and the solid material, which is fitted through the second slot and can be pulled back and forth according to the length of the second slot;

(j) a means for pulling the first slider to transfer the solid material from one chamber to the next chamber;

(k) a means for pulling the second slider to agitate the liquid reactants and the solid material; and

(l) a means for agitating the liquid reactants and the solid material in the plural chambers.

In the present invention, depending on the kinds of solid material, the various biological samples can be applied over isolation of nucleic acid or biological materials. The various biological samples are blood, bacteria, cultured cells, polymerase chain Reaction (PCR) samples, DNA sequencing samples, DNA-containing agarose product and other biological reaction samples.

In the present invention, the examples of the solid material absorbing or binding nucleic acid or biological materials are silica, glass fiber, anion exchange resins, natural magnetic bead, modified magnetic bead, Sephadex, Sepharose and Sephacryl. Some solid material such as silica, glass fibers, anion exchange resins, Sephadex, Sephacryl and Sepharose have the binding ability to nucleic acid or biological

material and can be settled down somewhat rapidly under gravity. The other solid material such as natural or modified magnetic bead have the binding ability to nucleic acid or biological materials depending on modification on surface and affinity to magnet. The feature of the solid material allows the collection of the solid material into the cavity of the slider.

The advantages over the conventional isolation of nucleic acid or biological materials are:

i) There is no need for tedious manual pipetting steps and therefore it is possible to eliminate the potential human error such as contamination and mishandling of the samples during the process;

ii) The risk of infection by potential infectious agents inside the sample such as virus and bacteria is greatly reduced since the isolation procedure is done in a closed cassette; and

iii) The number of samples analyzed at one time can be varied depending on the sets of chamber inside a cassette.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other object, feature, and advantage of the present invention will be apparent from the following detailed description of the preferred embodiments of the invention in conjunction with the accompanying drawings:

FIG. 1 is a cross sectional view illustrating sequential steps of isolating a nucleic or biological materials using an apparatus according

to the present invention;

FIG. 2 is a top view of an apparatus according to the present invention in which a cassette contains eight sets of plural chambers;

FIG. 3 is a cross sectional view of an apparatus according to one example of the present invention; and

FIG. 4 is a cross sectional view of an apparatus according to another example of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides an apparatus for isolating nucleic acid or biological material from various biological samples. The feature in the present invention is that solid material such as silica, glass fibers, exchange resin, modified magnetic beads and etc. is used for binding or absorbing nucleic acid or biological materials to remove the use of organic solvents and the centrifugation step. To eliminate the pipetting steps, a slider is applied for efficient movement of solid material binding nucleic acid or biological materials.

FIG. 1 is a cross sectional view illustrating sequential steps of isolating a nucleic or biological materials using an apparatus according to a preferred embodiment of the present invention.

As shown in **FIG. 1**, a cassette **100** comprises four chambers **102a, 102b, 102c** and **102d** with respective input holes **101a, 101b, 101c** and **101d**. The four chambers **102a, 102b, 102c** and **102d** are connected with one another with a slot which is fitted with a slider **105**. Although the

slider **105** can be pulled back and forth according to the length of the slot, the slider **105** fits into the slot very tightly so that the spaces of the chambers **102a, 102b, 102c** and **102d** are separated and the liquid reactants **103a, 103b, 103c** and **103d** which is added into the chambers **102a, 102b, 102c** and **102d** are sustained respectively. The slider **105** has a small cavity **106** that is capable to settle or collect a solid material at one of its end parts. The depth, area and shape of the cavity can be varied depending on the kind of used solid material and the shape of the chambers **102a, 102b, 102c** and **102d**. Preferably, the cross sectional shape of the cavity **106** is the same with that of the chambers **102a, 102b, 102c** and **102d**.

The liquid reactants **103a, 103b, 103c** and **103d** in each chambers **102a, 102b, 102c** and **102d** can be various buffers such as lysis buffer, ethanol elution buffer, distilled water and etc. which is needed in the process of isolating nucleic acid or biological materials.

In case of isolating nucleic acid from blood using magnetic bead as solid material, the process starts with an addition of blood into the chamber **102a** that already contains lysis buffer **103a** and a magnetic bead **104**. To facilitate the lysis reaction, the whole cassette **100** is shaken, for example, back and forth, or in seesawing or rotating motion in conjunction with motioning system (not shown). After shaking of the cassette **100**, a magnet **107** is applied to collect the DNA-carrying magnetic bead **104** into the cavity **106** of the slider **105** as shown in **FIG. 1**(a).

To transfer the magnetic bead **104** to the next chamber **102b**, the slider **105** is pulled manually or by automatic pulling system driven by a motor (not shown) and the cavity **106** is located in the bottom of the chamber **102b** as shown in **FIG. 1**(b).

After that, for washing the magnetic bead **104**, the whole cassette **100** is shaken, for example, back and forth or in seesawing or rotating motion for agitating the liquid reactants **103b** and the magnetic bead **104**, and then the magnet **107** is applied to collect the solid material **104**. The procedure of pulling the slider **105**, shaking of the cassette **100** and applying of the magnet **107** is repeated in the step of **FIG. 1**(c) and (d).

In **FIG. 1**(d), the liquid reactant **103d** is an elution buffer to elute the nucleic acid from magnetic bead **104** and finally the liquid reactant **103d** containing nucleic acid in the last chamber **102d** is collected by pipetting through an input hole **101d**.

Although the input hole **101a**, **101b**, **101c** and **101d** was shown up in a simplified format in **FIG. 1**, other input methods such as a self-sealing piercable diaphragm, and etc. can be used. Also the input holes **101a**, **101b**, **101c** and **101d** can be equipped with a sliding lid to avoid flowing of the liquid reactants **103a**, **103b**, **103c** and **103d**. In **FIG. 1**, the step of magnet **107** application to collect the magnetic bead **104** can be omitted if the other type of solid materials such as silica and resins that are easily settled down by gravity is used instead of the magnetic bead **104**.

In **FIG. 2**, a cassette **201** contains eight sets of the chambers **202** for isolating nucleic acid or biological materials from eight different

samples. A slider **205** can be pulled according to the direction of the arrow. Although the eight sets of the chambers are shown, the number of the chamber set can be varied from 1 to 20,000. The number of chamber set can be increased by preferably reducing the size of each chamber or enlarging the size of the cassette. The shape of each chamber can be varied within limited area of the chamber.

FIG. 3 is a cross sectional view of an apparatus according to one embodiment of the present invention.

In the present embodiment, the apparatus is explained focused on a means for pulling the slider, a means for generating magnetic force, and a means for agitating the liquid reactants and the solid material.

Agitation of the liquid reactants and the solid material is achieved by a seesawing part **311** that holds and seesaws the cassette.

The means for pulling the slider comprises a belt **314**, a hook **313** that fixes the slider to the belt **314**, a pivoting point **312** to hold one side of the belt **314**, and a motor **315** that drives the belt **314**. Accordingly, the slider is pulled by driving the motor **315**.

The means for generating magnetic force comprises a magnet **318**, a belt **317**, a pivoting point **316** to hold one side of the belt **317** and a motor **319** to drives the belt. Thereby, a magnet can be moved according to the movement of magnetic solid material.

Addition to that, a means for controlling the temperature of the plural chambers was added. The means for controlling the temperature is a heater **320** which is operated by electrical power and controlled by a

censor **321**.

These four parts can be coordinated by programmable software to prosecute the isolation of nucleic acid or biological materials appropriately.

FIG. 4 is a cross sectional view of an apparatus according to the other embodiment of the present invention. The main difference of the apparatus in **FIG. 4** from the apparatus in **FIG. 3** is addition of a second slider **408** on the upper part of a cassette **400** instead of seesawing part, and a system to pull the cassette body **400** and a cassette holder **408** instead of the system to pull a slider **405** and a magnet **407**. To simplify the figure, the input holes are omitted. The second slider **408** is a flat plate having multi number of vertical intruder **410**. The purpose of the second slider **408** is to agitate the liquid reactants and the solid material in the plural chambers. The second slider **408** has an advantage especially for the case that the mixing reaction is well not achieved by shaking the cassette since the liquid reactants have high surface tension. The liquid reactants and the solid material are agitated by pulling the second slider **408** back and forth repeatedly. A means for pulling the second slider **408** comprises a belt (not shown), a hook **413** that fixes the slider to the belt, and a motor (not shown) that drives the belt.

Movement of the solid material on the cavity is achieved by moving the cassette **400**, the cassette holder **409**, and the second slider **408** using a belt **411** connected to the cassette holder **409**, and a motor **412** driving the belt **411**.

What I claim is;

1. An apparatus for isolating nucleic acid or biological material from biological sample using a solid material which carries or absorbs nucleic acid or biological material, the apparatus comprising:

(a) a cassette in which plural chambers and input holes into the respective chambers are formed and the plural chambers are connected through a slot which is formed in the bottom of the chambers;

(b) a slider which is fitted through the slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the slider can be pulled back and forth according to the length of the slot;

(c) a cavity which is formed on the chamber side of the slider to settle or collect a solid material in the chamber;

(d) a means for pulling the slider to transfer the solid material from one chamber to the next chamber; and

(e) a means for agitating the liquid reactants and the solid material in the plural chambers.

2. The apparatus according to the claim 1, wherein the slider is in a shape of flat plate in which the cavity is formed at one of its end parts.

3. The apparatus according to the claim 1, wherein the solid material is selected from the group consisting of silica, glass fiber, anion exchange resins, Sephadex, Sepharose, Sephacryl and magnetic bead.

4. The apparatus according to the claim 1, further comprising a means for generating magnetic force which is located below the slider to settle or collect a magnetic solid material.
5. The apparatus according to the claim 1, further comprising a means for controlling a temperature of the plural chambers.
6. The apparatus according to the claim 1, wherein the operation of the whole apparatus is automated.
7. The apparatus according to the claim 1, wherein the agitation of the liquid reactants and the solid material is achieved by shaking the cassette in the seesawing motion.
8. An apparatus for isolating nucleic acid or biological material from biological sample using a solid material which carries or absorbs nucleic acid or biological material, the apparatus comprising:
   (a) a cassette in which plural chambers and input holes into the respective chambers are formed, the bottoms of the plural chambers are connected through a first slot and the tops of the plural chambers are connected through a second slot;
   (b) a first slider which is fitted through the first slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the first slider can be pulled back and forth according to the length of the first slot;
   (c) a cavity which is formed on the chamber side of the first slider to settle or collect a solid material in the chamber;
   (d) a second slider having plural intruders toward the plural

chambers to agitate the liquid reactants and the solid material, which is fitted through the second slot and can be pulled back and forth according to the length of the second slot;

(e) a means for pulling the first slider to transfer the solid material from one chamber to the next chamber;

(f) a means for pulling the second slider to agitate the liquid reactants and the solid material; and

(g) a means for agitating the liquid reactants and the solid material in the plural chambers.

9. The apparatus according to the claim 1, wherein the first slider is in a shape of flat plate in which the cavity is formed at one of its end parts.

10. The apparatus according to the claim 1, wherein the solid material is selected from the group consisting of silica, glass fiber, anion exchange resins, Sephadex, Sepharose, Sephacryl and magnetic bead.

11. The apparatus according to the claim 1, further comprising a means for generating magnetic force which is located below the slider to settle or collect a magnetic solid material.

12. The apparatus according to the claim 1, further comprising a means for controlling a temperature of the plural chambers.

13. The apparatus according to the claim 1, wherein the operation of the whole apparatus is automated.

14. The apparatus according to the claim 1, wherein the agitation of the liquid reactants and the solid material is achieved by pulling back

and forth according to the length of the second slot.

FIG. 1

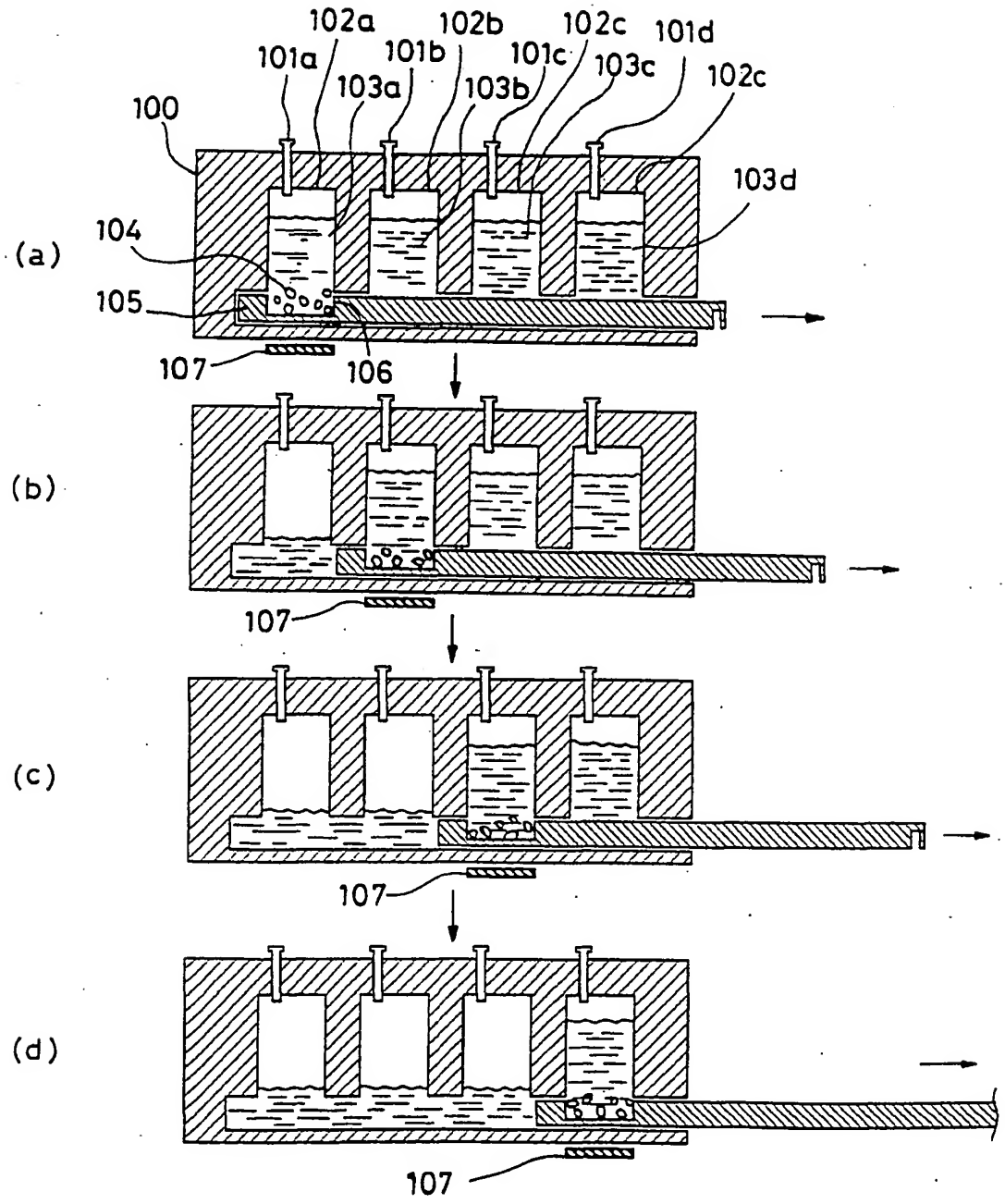
(a)
(b)
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FIG. 2

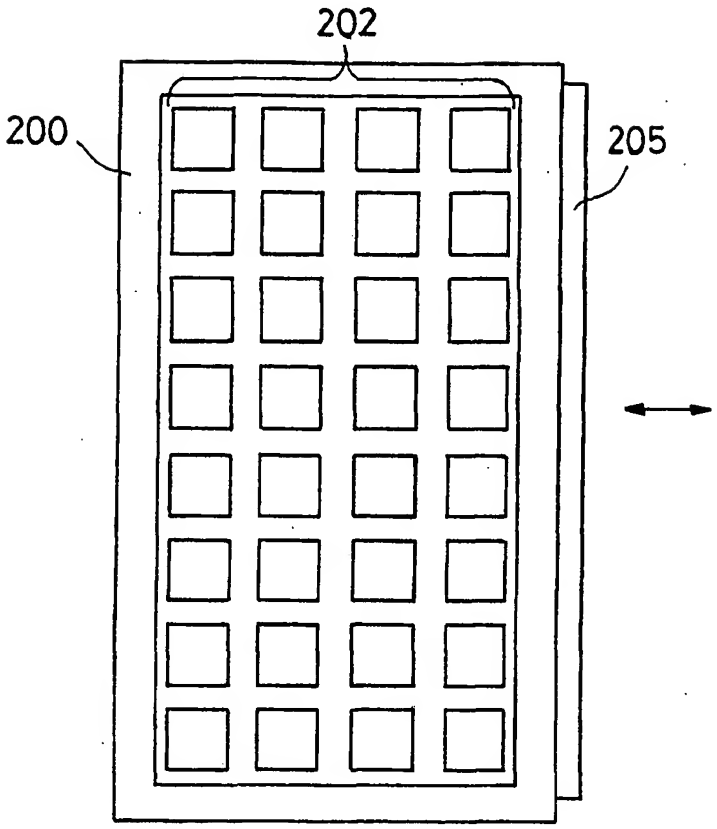


FIG. 3

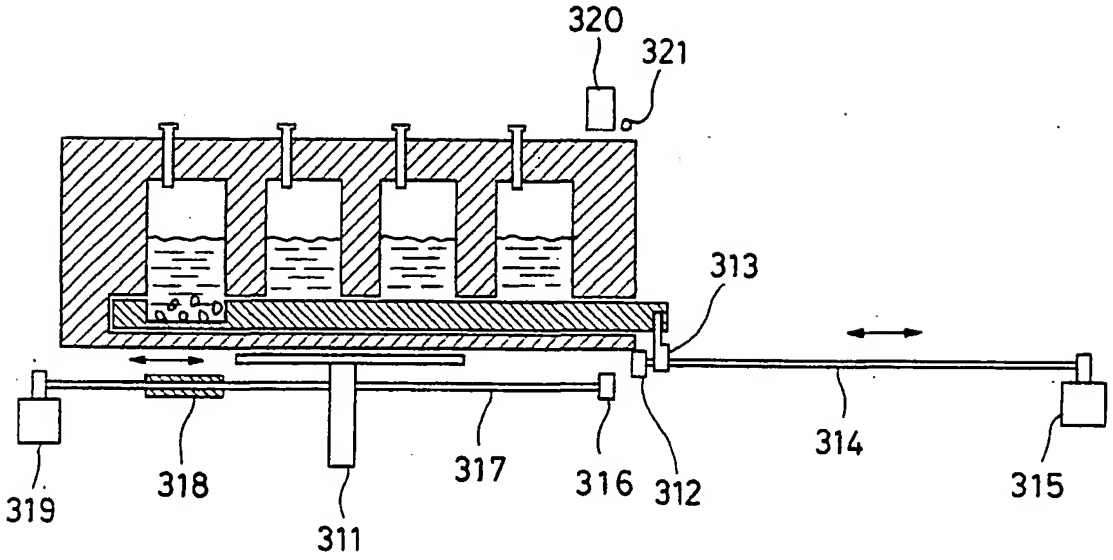
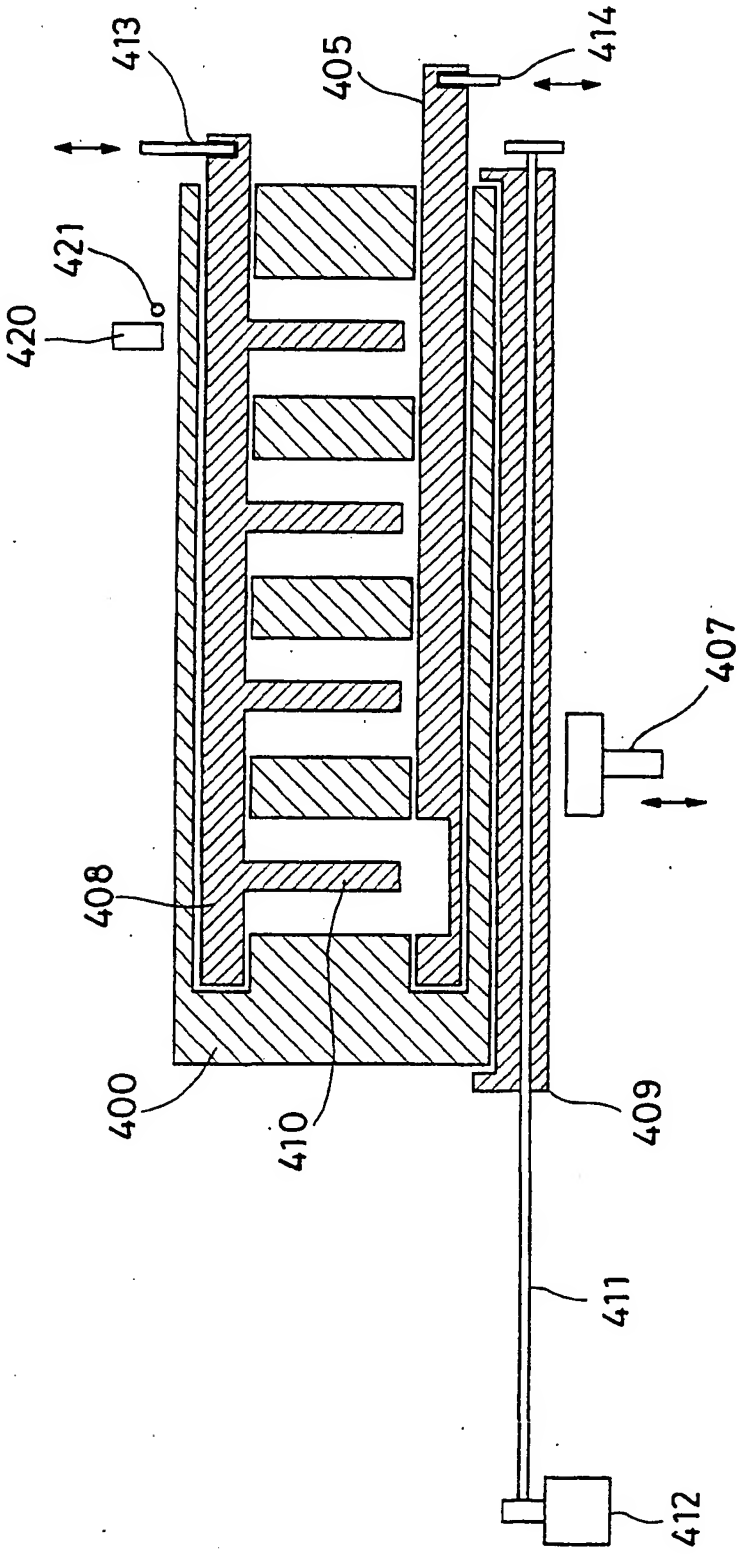


FIG. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/00153

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12Q 1/68, C07H 21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)

IPC7 C12Q 1/68, C07H 21/00

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Korean Patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)

USPAT, FPD, PAJ "(apparatus or device) and (purif* or extract* or isolat*) and nucleic"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0487028 A2 (SHIMADZU CO.) 27 MAY 1992	1 - 14
A	WO 97/08547 A1 (THEOBALD SMITH RESEARCH INSTITUTE, INC) 06 MARCH 1997	1 - 14
A	EP 0933132 A2 (TOYO BOSEKI KABUSHIKI KAISHA) 04 AUGUST 1999	1 - 14

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 NOVEMBER 2001 (14.11.2001)	19 NOVEMBER 2001 (19.11.2001)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR01/00153

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0487028 A2	27.05.92	JP4187077 A	03.07.92
WO 97/08547 A1	06.03.97	US 5804684 A AU 6857596 A	08.09.98 19.03.97